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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
C07D 205/12, C07C 229/48 C12P 41/00, 13/04	A1	WO 92/18477
		(43) International Publication Date: 29 October 1992 (29.10.92)

(21) International Application Number: PCT/GB92/00731

(22) International Filing Date: 21 April 1992 (21.04.92)

(30) Priority data:
9108384.0 19 April 1991 (19.04.91) GB

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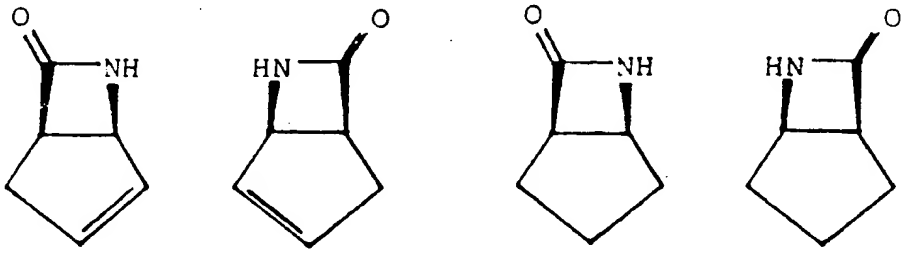
(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: AZABICYCLOHEPTANONE



(Ia) (Ib) (IIa) (IIb)

(57) Abstract

The individual enantiomers of the bicyclic β-lactam compounds (Ia), (Ib), (IIa) or (IIb), optionally substituted by non-interfering substituent(s). The novel enantiomers can be obtained by biotransformation. The Ia or IIa compounds can be used for the synthesis of chiral cis-pentacin.

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AZABICYCLOHEPTANONE

Field of the Invention

This invention relates to bicycloheptanes, their preparation, and their use as chiral synthons.

Background of the Invention

Oki et al, J. Antibiotics 42 (1989) 1756, disclose the anti-fungal microbial antibiotic cis-pentacin, of formula III (see below).

This compound can be prepared by hydrolysis of the ß-lactam (IIa) which may in turn be made by catalytic hydrogenation of the unsaturated ß-lactam (Ia).

A preparation of III in optically-active form is described by Korushi et al, J. Antibiotics 42 (1989) 1749, involving repeated crystallisation of the (+)-dehydro abietylamine salt formed from the racemate. It is not expected that this represents an economic method of making more than gram quantities of the optically-active antibiotic.

EP-A-0424064 describes the enantioselective hydrolysis of the γ-lactam 2-azabicyclo[2.2.1]hept-5-en-3-one, utilising enzymatic activity available in deposits NCIMB 40213 and 40249. See also Taylor et al, J. Chem. Soc. Chem. Comm. (1990) 1120. As described by Evans et al, J. Chem. Soc. Perkin Trans. I (1991) 656, the cells used for that γ-lactam are apparently intolerant of structural variation.

Summary of the Invention

This invention concerns the enantiomers of azabicycloheptane derivatives of formulae Ia, Ib, IIa and IIb which, in optically pure form, are novel compounds. They may each be provided substantially free of other enantiomers.

The novel compounds are useful as intermediates to optically-pure biologically-active compounds; in particular, preparation of cis-pentacin from an optically-pure ß-lactam precursor (Ia) or (IIa) is advantageous.

Description of the Invention

An important discovery behind the present invention is that material of biological origin will react selectively on racemates of the ß-lactam (Ia + Ib) or (IIa + IIb) to give one enantiomer as untransformed lactam recoverable by solvent extraction, and the other as hydrolysis product, i.e. the amino-acid; the amino-acid is retained in aqueous solution upon extraction with solvent, but may be isolated, if required, for instance by adsorption on an ion-exchange resin.

Suitable activities for the enantioselective transformation are described in EP-A-0424064, e.g. those present in certain novel wild-type isolates of the genera Pseudomonas, Alcaligenes, Arthrobacter, Brevibacterium, Nocardia, Rhodococcus and Corynebacterium, whilst not being limited to isolates of these genera. Selection for these activities may be conducted in the presence of compounds containing one or more N-acyl substituents. If necessary, elevated levels of activity may be produced by growth of cells in the presence of such compounds. Particular examples of suitable activity are those produced maximally active in cells of the unique strains ENZA-20 and Rhodococcus sp. ENZA-1, the latter when cultivated in a suitable medium in the presence of N-acetyl-L-phenylalanine or N-acetyl-D,L-phenylalanine.

Rhodococcus sp. ENZA-1 was isolated from soil samples by enrichment culture in mineral salts medium containing N-acetyl-L-phenylalanine as the sole source of carbon and energy. The isolate has been deposited at the NCIMB in Aberdeen, on 17.10.89. The accession number is NCIMB 40213.

Although the novel biotransformation is analogous to that described previously, and in EP-A-0424064, the enantioselective hydrolysis of the γ-lactam 2-azabicyclo-[2.2.1]hept-5-en-3-one, efficient transformation of the ß-lactam (Ia + Ib) was unexpected, considering that the

cells used for the γ-lactam seemed intolerant of structural variation.

The racemic unsaturated ß-lactam (Ia + Ib) is made by the known method of [2+2]cycloaddition of chlorosulphonyl isocyanate onto cyclopentadiene, followed by hydrolytic removal of the chlorosulphonyl function with sodium sulphate. This synthesis is described by Malpass et al, J.Chem.Soc.Perkins Trans. I (1977) 874. For the preparation of cis-pentacin it can be advantageous to proceed via the racemic saturated ß-lactam (IIa + IIb), for this can be made in an efficient cycloaddition between cyclopentene and chlorosulphonyl isocyanate.

The enantiomers of formulae Ib and IIb are useful synthons in the preparation of ß-lactams, cis-pentacin analogues and amino-acid isosteres.

The compounds of the invention may be substituted, if desired, by non-interfering substituents, i.e. substituents that do not affect the biotransformation. Examples of substituents (if present) are methyl, ethyl, n-butyl, OH, Cl, Br, F, CF_3 and N_3. The total number of C atoms in the substituent(s) will not usually exceed 8 or, more usually, 4.

The following Examples illustrate the invention. In each case, the ee value obtained exceeds 99%.

Example 1 Whole cell resolution of 6-azabicyclo[3.2.0]hept-3-en-7-one

Rhodococcus equi NCIB 40213 (ENZA-1; 700 mg paste) was suspended in phosphate buffer (0.05 M; pH 7) and racemic 6-azabicyclo[3.2.0]hept-3-en-7-one (340 mg, 3.12 mmol) was added. The mixture was stirred at ambient temperature for 142 h after which the cells were removed by centrifugation. The supernatant was extracted with dichloromethane (4 x 100 ml) and the combined organic layers were dried ($MgSO_4$) and concentrated. The recovered lactam (197 mg) was reincubated with ENZA-1 (280 mg) in buffer (36 ml) for a further 170 h, then recovered as above. Column chromatography over silica using acetate as eluent gave

(1R,2S)-(+)-6-azabicyclo[3.2.0]hept-3-en-7-one (137 mg, 40%) as a white solid; m.p. 76-77°C; $[\alpha]^{20}_D$ 37 (c = 0.4, $CHCl_3$); νmax ($CHCl_3$) 3414 and 1754 cm^{-1}; δ_H ($CDCl_3$) 6.25 (1 H, br s, NH), 6.12-5.85 (2 H, m, 3-H and 4-H), 4.59-4.42 (1 H, m, 5-H), 3.85 (1 H, ddd, J 9.8, 3.5, 3.5 Hz, 1-H) and 2.90-2.25 (2 H,m, 2-H_2).

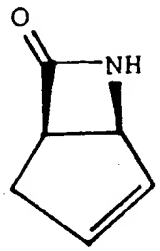
The strain ENZA-1 thus gave, from the racemic ß-lactam (Ia + Ib), the [1R,5S]-(+)-lactam (Ia) in >99% ee and 40% yield together with an amino-acid of opposite configuration which was isolated as its methyl ester, acetamide, in 96% ee. The recovered lactam (Ia) was of the correct stereochemistry for conversion into (-)-cis-pentacin.

Example 2 Hydrogenation of (1R,2S)-(+)-6-azabicyclo[3.2.0]-hept-3-en-7-one

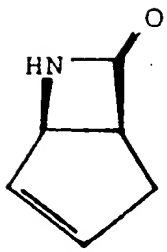
The title lactam (57 mg, 0.52 mmol) in ethyl acetate (3 ml) was added to a suspension of 10% palladium on charcoal (8 mg) in ethyl acetate (2 ml). The mixture was stirred under hydrogen at ambient temperature for 1½ h. when Hc indicated complete reaction (on silica, eluent ethyl acetate, $KMnO_4$ development, product has R_F 0.38, starting material has R_F 0.45). The mixture was filtered through Celite and the filtrate concentrated to a colourless oil (55 mg 95%) which solidifies below room temperature. It was pure by GC and ^1H NMR; $[\alpha]_D$ -38 (c = 1.1, $CHCl_3$).

Example 3 cis-pentacin

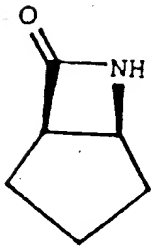
The reduced lactam from Example 2 (53 mg, 0.48 mmol) was stirred in 2 M hydrochloric acid (6 ml) at ambient temperature for 1 h when Hc indicated complete reaction (silica, ethyl acetate:methanol 4:1, $KMnO_4$ development). The mixture was loaded onto a Dowex (H^+) column and eluted with water followed by 1 M aqueous ammonia. Fractions containing product were concentrated to give cis-pentacin [(1R,2S)-2-aminocyclopentane-1-carboxylic acid] (60 mg. 97%) as an off-white solid $[\alpha]_D$ -8 (c = 1, H_2O).



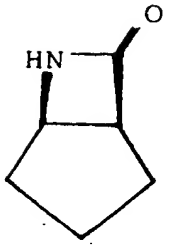
Ia



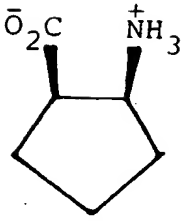
Ib



IIa



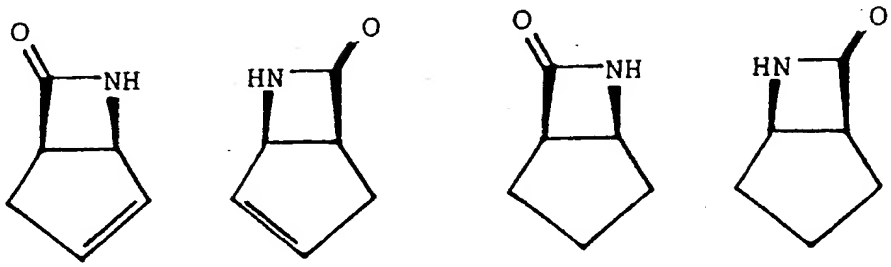
IIb



III

CLAIMS

1. The individual enantiomers of the bicyclic ß-lactam compounds (Ia), (Ib), (IIa) or (IIb),



Ia Ib IIa IIb

optionally substituted by non-interfering substituent(s).

2. A process for the resolution of a mixture enantiomers of bicyclic ß-lactams (Ia + Ib) or (IIa + IIb) as defined in claim 1, by means of a biocatalyst derived from bacterial whole cells and characterised by its ability to hydrolyse only one enantiomer of the mixture, to give the corresponding ring-opened amino-acid.

3. Use of an enantiomer (Ia) or (IIa) as defined in claim 1, for the synthesis of the anti-fungal antibiotic cis-pentacin.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/00731

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D205/12; C07C229/48; C12P41/00; C12P13/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; C07C ; C12P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	THE JOURNAL OF ANTIBIOTICS vol. 42, no. 12, 1989, pages 1749 - 1755; KONISHI ET AL.: 'CISPENTACIN, A NEW ANTIFUNGAL ANTIBIOTIC.' cited in the application see the whole article, especially page 1753, sheme 2	1-3
A	JOURNAL OF THE CHEMICAL SOCIETY PERKIN TRANSACTIONS 1 no. 3, March 1991, pages 656 - 657; C. EVANS ET AL: 'SYNTHESIS OF EITHER ENANTIOMER DF CIS-3-AMINOCYCLOPENTANECARBOXYLIC ACID FROM BOTH ENANTIOMERS OF RACEMIC 2-AZABICYCLO (2.2.1) HEPT-5-EN-3 ONE.' cited in the application see the whole article	1-3

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° Special categories of cited documents : [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 JUNE 1992	10. 07. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	CHOULY J.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	TETRAHEDRON LETTERS no. 27, 1972, pages 2793 - 2796; H. REHLING ET AL.: 'CIRCULARDICHROISMUS UND ABSOLUTE KONFIGURATION VON BETA-LACTAMEN' see the whole article, especially page 2795, table 2, nr. 13	1
P,X	JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1 no. 9, September 1991, pages 2276 - 2277; C. EVANS ET AL.: 'WHOLE CELL CATALYSED KINETIC RESOLUTION OF 6-AZABICYCLO(3.2.0)HEP-3-EN-7-ONE: SYNTHESIS OF (-)-CISPENTACIN (FR 109615).' see the whole article	1-3
P,A	EP,A,0 424 064 (ENZYMATIX LTD.) 24 April 1991 cited in the application see claims	1-3

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9200731 SA 58488

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 30/06/92
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0424064	24-04-91	JP-A- 3218380	25-09-91

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82